

AMENDMENTS TO THE CLAIMS:

This listing of the claims will replace all prior versions, and listings, of claims in the present application.

LISTING OF THE CLAIMS:

31. (Currently amended) A method of isolating at least one anti-ligand to at least one target ligand comprising the steps of:

- (i) providing a library of anti-ligands;
- (ii) providing ~~an amount of a first subtractor ligand~~ a first population of ligands comprising a ligand fixed to or incorporated in a subtractor ligand construct;
- (iii) providing ~~an amount of a second target ligand~~ a second population of ligands comprising the same ligand as step (ii), fixed to or incorporated in a target ligand construct;
- (iv) determining amounts of ~~first subtractor~~ ligand construct and ~~second target ligands~~ ligand construct using one or more equations derived from the universal law of mass

action
$$\frac{[C]^c [d]^d}{[A]^a [B]^b} = K_{eq},$$

where:

A, B, C & D = are the participants in the reaction (reactants and products)

a, b, c, & d = the coefficients necessary for a balanced chemical equation

so as to permit isolation of at least one anti-ligand to at least one target ligand;

- (v) providing the amount of the ~~first subtractor ligand~~ construct determined in step (iv);
- (vi) providing the amount of the ~~second target ligand~~ construct determined in step (iv);
- (vii) providing separation means for isolating anti-ligand bound to the ~~second target ligand construct~~ from anti-ligand bound to the ~~first subtractor ligand construct~~, wherein said separation means for the subtractor and target ligand constructs can be either the same or different;

(viii) exposing the library of (i) to the ligands of (v) and (vi) to permit binding of anti-ligands to ligands; and

(ix) isolating the anti-ligand bound to the ~~second~~ ligand fixed to or incorporated in the target ligand construct ~~with using~~ the separation means.

Claim 32-33 (Canceled)

34. (Currently amended) A method as claimed in ~~one of claims claim~~ claim 31, 32 or 33 comprising a further step of releasing the anti-ligand from the ~~second target~~ ligand.

35. (Currently amended) A method as claimed in claim 31 ~~or 32~~ whereby steps (ii) to (ix) are repeated one or more times.

36. (Currently amended) A method as claimed in claim 31 ~~or 32~~ wherein the amount of one of the ~~first~~ subtractor ligand construct or ~~second~~ target ligand construct is provided in excess of the amount of the other of the ~~first~~ subtractor ligand construct or ~~second~~ the target ligand construct.

37. (Previously presented) A method as claimed in claim 36 where the excess of ligand is of 10 to 100 fold.

38. (Currently amended) A method as claimed in ~~claims claim~~ claim 31 or 32 wherein the equation of (iv) is

$$bA = \frac{(A + T + (K_d)x(CxV))}{2} - \sqrt{\frac{(A + T + (K_d)x(CxV))^2}{4} - AxT}$$

where

bA = Bound anti-ligand

A = Total number of anti-ligand

T = Total number of ligands

C = Avogadro's constant (6.022×10^{23} particles/mole)

V = Reaction volume (litres)

K_d = Equilibrium dissociation constant.

39. (Currently amended) A method as claimed in claims 31 ~~or 32~~ wherein the equation of (iv) is:

$$bA = \left\{ \frac{(A + T + (K_d)x(CxV))}{2} - \sqrt{\frac{(A + T + (K_d)x(CxV))^2}{4} - AxT} \right\} x \left\{ \frac{(T_p x C_p)}{((T_p x C_p) + (T_s x C_s))} \right\}$$

where

bA_p = Bound anti-ligand

T_p = The number of ligands on C_p

T_s = The number of ligands on C_s

C_p = The number of target ligand constructs

C_s = The number of subtractor ligand constructs

A = Total number of anti-ligand

T = Total number of ligands

C = Avogadro's constant (6.022×10^{23} particles/mole)

V = Reaction volume (litres)

K_d = Equilibrium dissociation constant.

40. (Currently amended) A method as claimed in claim 31 ~~or 32~~ wherein the separation means are selected from at least one of a solid support, cell membrane and/or portions thereof, synthetic membrane, beads, chemical tags and free ligand.

41. (Previously presented) A method as claimed in claim 40 whereby the separation means are cell membranes and/or portions thereof.

42. (Currently amended) A method as claimed in claim 41 whereby the ~~first~~ subtractor and ~~second~~ target ligands are fixed to and/or incorporated within separate cell membranes and/or portions thereof.

43. (Currently amended) A method as claimed in ~~claims~~ claim 31 ~~or 32~~ whereby the separation means of the ~~first~~ subtractor and ~~second~~ target ligand constructs have a different density.

44. (Currently amended) A method as claimed in claim 43 wherein the separation means

of the ~~first~~ subtractor ligand construct is of a lower density than the separation means of the ~~second~~ target ligand construct.

45. (Currently amended) A method as claimed in claim 44 wherein the separation means of the ~~first~~ subtractor ligand construct is a membrane vesicle.

46. (Currently amended) A method as claimed in claim 44 wherein the separation means of the ~~second~~ target ligand construct is a whole cell membrane.

47. (Currently amended) A method as claimed in claim 31 ~~or 32~~ whereby ~~the isolation of anti-ligand bound to second target ligand~~ step (ix) is performed by at least one of density centrifugation, solid support sequestration, magnetic bead sequestration, chemical tag binding and aqueous phase partitioning.

48. (Previously presented) A method as claimed in claim 47 whereby the isolation step is performed by density centrifugation.

49. (Previously presented) A method as claimed in claim 48 wherein the density centrifugation is performed using a sucrose-polymer gradient.

50. (Currently amended) A method as claimed in claim 31 ~~or 32~~ wherein the library of step (i) is a display library comprising a plurality of library members which display anti-ligands.

51. (Previously presented) A method as claimed in claim 50 wherein the library is a phage display library.

52. (Currently amended) A method as claimed in claim 31 ~~or 32~~ wherein the subtractor and target ligands are independently at least one from antigens; receptor ligands; and enzyme targets that comprise at least one selected from carbohydrate; protein; peptide; lipid; polynucleotide; inorganic molecules and conjugated molecules.

53. (Currently amended) A method as claimed in claim 31 ~~or 32~~ wherein the library of anti-ligands is composed of at least one selected from antibodies, and antigen binding variants, derivatives or fragments thereof; scaffold molecules with engineered variable surfaces; receptors; and enzymes.

54. (Currently amended) A method as claimed in claim 31 ~~or 32~~ comprising a further step of exposing the ligand and its separation means to a stimulus which influences the expression of target ligands on said ligand constructs.

Claims 55-59 (Canceled)